

mRNA preparation

MW Maaïke Welling

Updated date: Feb 20, 2020

 An abbreviated version of this protocol was published in eLIFE in Jan 2019

Primed Track, high-fidelity lineage tracing in mouse pre-implantation embryos using primed conversion of photoconvertible proteins

DOI: 10.7554/eLife.44491

Detailed protocol

mRNA preparation

Reagents:

- pCS2+-H2B-prmEosFP plasmid DNA containing a SP6 RNA polymerase site
- NotI restriction enzyme (NEB)
- 0.5M EDTA
- 3M NaAc
- 100% EtOH
- SP6 mMessage mMachine Kit (ThermoFisher)
- Poly-A-tailing kit (ThermoFisher)
- RNeasy kit (Qiagen)

Plasmid Linearization

1. Linearize 5ug of plasmid DNA with a restriction enzyme downstream of the insert that needs to be transcribed (NotI for pCS2+-H2B-pr-mEosFP) in 1x NEBuffer 3.1 for 1 hour at 37 degrees.
2. Terminate the restriction digest by adding:
 - 1/20th volume 0.5M EDTA
 - 1/10th volume 3M NaAc
 - 2 volumes of EtOH
3. Mix and incubate at -20 degrees for 15-30 minutes.
4. Spin down the DNA for 15 minutes at high speed.
5. Remove supernatant and resuspend the pellet in TE buffer to a final concentration of 0.5-1ug/ul.

In vitro mRNA synthesis & Poly-A-tailing

1. Follow the manufacturer's instructions of the SP6 mMESSAGE MACHINE kit for mRNA synthesis. Start with 1ug of linearized plasmid DNA.
2. Incubate the reaction for 2 hours at 37 degrees.
3. Add 1ul TURBO DNase provided with the kit and incubate for 15 minutes at 37 degrees to remove template DNA.
4. Use the complete DNase-treated mMESSAGE mACHINE reaction for Poly-A-Tailing and add reagents of the Poly-A-tailing kit according to the manufacturer's instructions.
5. Incubate reaction for 1 hour at 37 degrees.

mRNA purification

1. Use the Qiagen RNeasy kit for purifying the in vitro transcribed mRNA by following the "RNA cleanup" instructions.
2. Elute RNA in 50ul nuclease free water

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Welling, M. (2020). mRNA preparation. Bio-protocol Preprint. [bio-protocol.org/prep217](https://doi.org/10.21203/rs.3.rs-1000000/v1).

2. Welling, M., Mohr, M. A., Ponti, A., Rullan Sabater, L., Boni, A., Kawamura, Y. K., Liberali, P., Peters, A. H., Pelczar, P. and Pantazis, P.(2019). Primed Track, high-fidelity lineage tracing in mouse pre-implantation embryos using primed conversion of photoconvertible proteins. eLIFE. DOI: [10.7554/eLife.44491](https://doi.org/10.7554/eLife.44491)

Copyright: Content may be subjected to copyright.